### **REMARKS**

Claims 12-14, 17 and 18 are amended. Claims 15, 31, 33 and 34 are cancelled. Claims 22-30 are withdrawn from consideration. Claims 12-14, 16-21, 32, 35 and 36 remain active and under consideration.

At the outset, Applicants wish to thank Examiner Marvich for the recent helpful and courteous discussion conducted with their U.S. representative, Mr. William Beaumont. The claims amended herewith and associated remarks are in accordance with that discussion, and are responsive to the Advisory Action of March 24, 2010.

#### Claim 36

Claim 36 is not an 'added new claim'. This claim corresponds to claim 4 of the PCT application, and to claim 15 of the Preliminary Amendment of this U.S. application. During prosecution, claim 15 was replaced by claim 36. Hence, claim 36 does not present new issues for consideration.

Further, claim 36 is readily distinguishable over the art of record. For example, and in contrast, Poquet et al (Journal of Bacteriology, 1998) merely describe a construct which contains a part of the sequence coding for ZitS. This reference clearly does not read on claim 36 which clearly excludes expression cassettes containing any part of the sequence encoding ZitS. MPEP 2173.05 (i) explicitly sets forth the acceptability of negative limitations in complying with 35 USC 112, second paragraph. Moreover, in this case, the negative limitation "wherein the

expression cassette does not contain any part of the sequence encoding the L. lactis ZitS protein" provides a clear distinction over Poquet et al as well as the two other references noted in Advisory Action.

## Claims 17 and 18

Claims 17 and 18 are amended pursuant to the recent discussion with Examiner Marvich. Hence, the previous objections to these claims is deemed moot.

# Sequence Listing For AAK06214

As noted previously, the protein sequence deposited under GenBank accession number AAK06214 was available in 2001. See the "Sequence Revision History" from NCBI (of record) which shows that protein AAK06214 was available as of February 9, 2001. The corresponding nucleotide sequence was also available then because the genome of Lactococcus lactis strain IL 1403 was also sequenced in 2001. See Bolotin et al., "The complete genome sequence of the lactic acid bacterium Lactococcus lactis ssp. Lactis IL1403", Genome Res., 2001 May 11(5):731-753. Further, the strain IL1403 is laboratory strain commonly used and widely

accessible.

Thus, Applicants again assert that one skilled in the art would have no difficulty in obtaining the polynucleotide sequence encoding the protein AAK06214, or in reproducing it by synthesis or by screening a DNA library of Lactococcus lactis strain IL1403 with probes and/or primers derived from sequence data available in GenBank database. Finally, after having obtained this sequence, the artisan would have no difficulty in incorporating the sequence into an expression cassette according to the claimed invention.

However, Applicants provide herewith a compliant sequence listing and CRF version as requested by the examiner. Further, attached Annex 1 shows the different update dates for sequence AAK06214. May 14, 2001 update (last update before the priority and filing dates of the application). Annex 2 shows the amino acid sequence of locus AAK06214 that was available at the time of filing. The February 26, 2010 update (Annex 3) gives the same sequence as may 14, 2001 update. See Annex 4.

Accordingly, in view of all of the above, it is believed that this application is now in condition for allowance. Favorable consideration to this effect is earnestly solicited.

Respectfully submitted,

William E. Beaumont

Reg. No. 30,996

Juneau Partners, PLLC Customer No.: 50438



# **Sequence Revision History**

My NCBI [? [Sign In] [Register]

**Books** 

PubMed

Nucleotide

**Protein** 

Genome

Structure **PMC**  Taxonomy

MIMO

AAK06214

Find (Accessions, GI numbers or Fasta style Seqlds)

-Go

Clear

About Entrez

Show difference between I and II as GenBank/GenPept

**Entrez** 

Search for Genes Entrez Gene provides gene-specific data for multiple taxa

Help | FAQ

Batch Entrez: Upload a file of GI or accession numbers to retrieve protein OF nucleotide sequences

Check sequence revision history

How to create WWW links to Entrez

LinkOut

Related resources BLAST Reference sequence project Entrez Gene Clusters of orthologous groups Protein reviews on the web

**Revision history for AAK06214** 

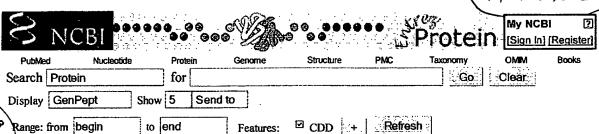
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| 12725171 | 1       | May 14 2001 12:46<br>PM | Dead   | 0 | 0  |
| 12725171 | 1       | Feb 9 2001 4:25 PM      | Dead   | 0 | 0  |

Accession AAK06214 was first seen at NCBI on Feb 9 2001 4:25 PM

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Last update: Thu, 17 Dec 2009 Rev. 178872

ANNEX



Features:

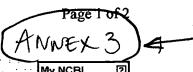
1: AAK06214. Reports zinc transport tr...[gi:12725171]

BLink, Related sequences, Identical proteins, Links

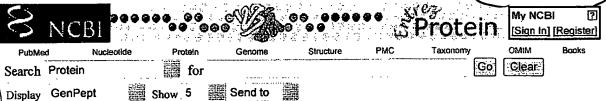
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REFERENCE
               (residues 1 to 145)
  AUTHORS
            Bolotin, A., Wincker, P., Mauger, S., Jaillon, O., Malarme, K.,
            Weissenbach, J., Ehrlich, S.D. and Sorokin, A.
  TITLE
            The complete genome sequence of the lactic acid bacterium
            Lactococcus lactis ssp. lactis IL1403
            Genome Res. 11 (5), 731-753 (2001)
  JOURNAL
   PUBMED
            11337471
REFERENCE
                (residues 1 to 145)
  AUTHORS
            Bolotin, A., Wincker, P., Mauger, S., Jaillon, O., Malarme, K.,
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  TITLE
            Direct Submission
  JOURNAL
            Submitted (09-JAN-2001) INRA, Genetique Microbienne, Domaine de
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            Method: conceptual translation supplied by author.
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Range: from begin







Features: CDD

Refresh

1: AAK06214. Reports zinc transport tr...[gi:12725171]

to :end

BLink, Related sequences, Identical proteins, Links

**Features** Sequence linear BCT 26-FEB-2009 145 aa AAK06214 LOCUS zinc transport transcriptional regulator [Lactococcus lactis subsp. DEFINITION lactis Il14031. ACCESSION AAK06214 en diguent sur 26/02/2010. AAK06214.1 GI:12725171 VERSION DBLINK Project: 72 DBSOURCE accession AE005176.1 KEYWORDS Lactococcus lactis subsp. lactis Il1403 SOURCE Lactococcus lactis subsp. lactis Il1403 ORGANISM Bacteria; Firmicutes; Lactobacillales; Streptococcace Lactococcus. REFERENCE (residues 1 to 145) Bolotin, A., Wincker, P., Mauger, S., Jaillon, O., Malarm **AUTHORS** Weissenbach, J., Ehrlich, S.D. and Sórokin, A. The complete genome sequence of the lactic acid bacterium TITLE Lactococcus lactis ssp. lactis IL1403 **JOURNAL** Genome Res. 11 (5), 731-753 (2001) 11337471 PUBMED REFERENCE (residues 1 to 145) **AUTHORS** Bolotin, A., Wincker, P., Mauger, S., Jaillon, O., Malarme, K., Weissenbach, J., Ehrlich, S.D. and Sorokin, A. TITLE Direct Submission JOURNAL Submitted (09-JAN-2001) INRA, Genetique Microbienne, Domaine de Vilvert, Jouy en Josas 78352, France Method: conceptual translation supplied by author. COMMENT FEATURES Location/Qualifiers source 1..145 /organism="Lactococcus lactis subsp. lactis Il1403" /strain="IL1403" /sub\_species="lactis" /db xref="taxon:272623" Protein 1..145 /product="zinc transport transcriptional regulator" 22..142 Region /region\_name="MarR" /note="Transcriptional regulators [Transcription]; COG1846" /db xref="CDD: 32031" 35..130 Region /region name="WHTH GntR" /note="Winged helix-turn-helix (WHTH) DNA-binding domain of the GntR family of transcriptional regulators; cl00088" /db xref="CDD: 173998" CDS 1..145 /gene="zitR" /locus tag="L168265" /coded by="complement (AE005176.1:2184820..2185257)" /note="EVIDENCE BY HOMOLOGY BIO09.08 Mark-family regulators. belongs to the MarR family. 48% identical to AdcR repressor of adc locus, affecting competence in

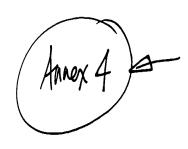
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> > Last update: Fri. 09 Apr 2010 Rev. 188411



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|                                   | 6214.1 AE006439 1:                       | 1 zinc transport transcription   | al regulator (Lact                     | ococcus lactis s                      | subsp. lactis Il1         | 403]         |
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